Ann & Robert H Lurie Children's Hospital of Chicago/ Northwestern University Feinberg School of Medicine/ Neonatology

Hyperoxia-induced lung engraftment of human cord blood monocytes in a humanized mouse model of bronchopulmonary dysplasia

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BACKGROUND

Bronchopulmonary dysplasia (BPD) is characterized by chronic lung injury secondary to high levels of oxygen during the postnatal period. Properties of the preterm infant during the perinatal period, such as the presence and function of stem and progenitor cells, may interact with postnatal exposures such as hyperoxia. Alveolar macrophages derived from cord blood-derived (fetal) monocytes play a vital role in lung injury and repair. Previous studies have demonstrated administration of human cord blood (CB) monocytes results in decreased alveolar damage due to hyperoxia exposure but fail to demonstrate engraftment of these cells in the lung of the animal model. The MISTRG mice are IL2RG and Rag2 deficient while expressing human GM-CSF, IL-3/GM-CSF, TPO, and a SIRPa transgene, making it a useful model to study the effects of hyperoxia and human CB monocyte engraftment.

HYPOTHESIS

Administration of human cord blood monocytes in MISTRG humanized mice ameliorate the BPD-like phenotype after exposure to hyperoxia.

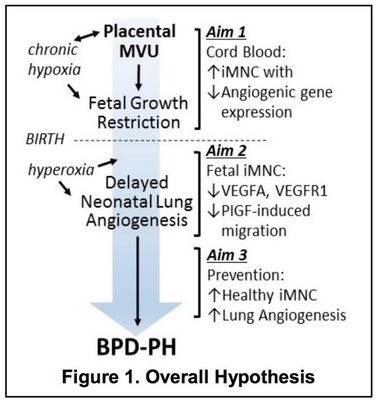


Figure 1. Overall Hypothesis

METHODS

- Collected human cord blood from births at Prentice Women's Hospital
- Processed human CB within 36 hours of delivery
- Isolated monocytes and CD34+ cells by magnetic-activated cell sorting (MACS)
- Performed intra-hepatic injections of sorted monocytes, isolated CD34+ cells, and PBS (sham) at Day 0-1 of life
- Exposed pups to normoxia (21% oxygen) or hyperoxia (85% oxygen) for 7 days
- Harvested lungs and livers and performed H&E staining and immunohistochemistry for human CD45
- Measured alveolar count and area in addition to quantifying human CD45 expression in the lung and liver

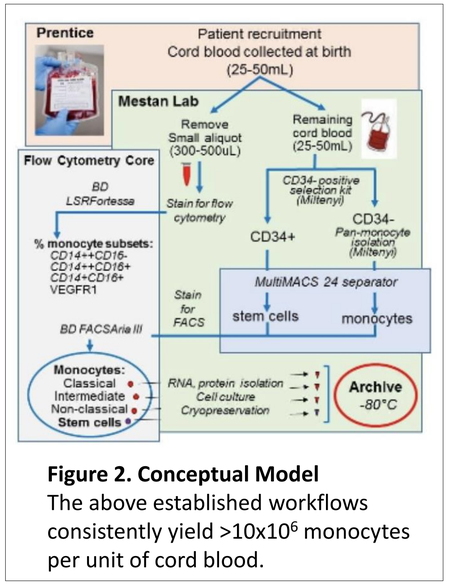
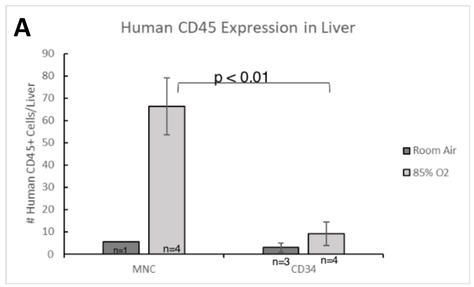


Figure 2. Conceptual Model
The above established workflows consistently yield >10x10^6 monocytes per unit of cord blood.

RESULTS

Figure 3. Cell Engraftment in the Liver



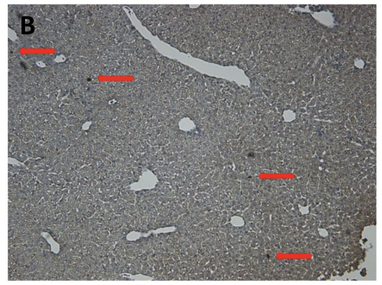
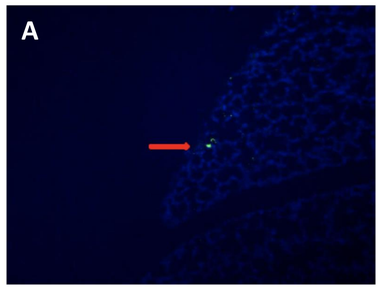
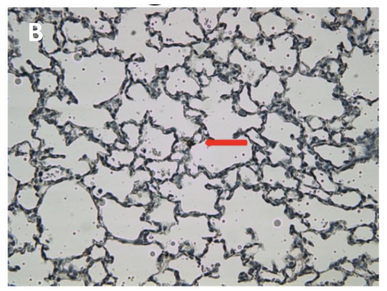


Figure 4. Cell Engraftment in the Lung

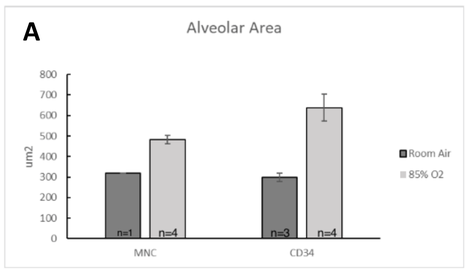


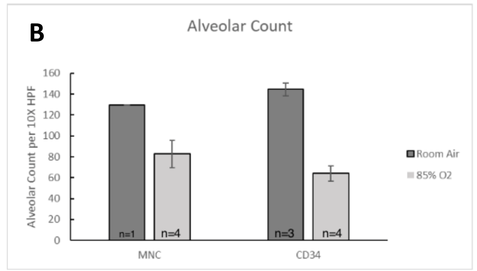


A. P7 Mouse Lung (Normoxia) – Human CD45 (Alexa488) and DAPI

B. P7 Mouse Lung (85% O2) – Human CD45 (DAB) Immunohistochemistry

Figure 5. Lung Histology





CONCLUSION

Intra-hepatic delivery of cord blood-derived monocytes leads to engraftment of human CD45+ cells in murine lungs, and may attenuate alveolar simplification due to hyperoxia. In the absence of CD34+ hematopoietic stem cells, the effects appear to be due to monocyte delivery from liver to lung, which is facilitated by human GM-CSF. Completion of the above studies, including quantification of engrafted human cells in the lungs and correlation with alveolar parameters, will demonstrate the extent to which CB monocytes may have a protective effect on neonatal lungs exposed to hyperoxia.

NEXT STEPS

1) Do the clinical characteristics of the patients (e.g., IUGR, chorioamnionitis, placental insufficiency) from which MNCs are derived influence engraftment and/or alveolar histology?
2) Investigate changes in engraftment and alveolar histology with different MNC subsets (classical, intermediate, non-classical).
3) Interaction of intrauterine growth restriction (Thromboxane A2 analog) and hyperoxia on MNC engraftment.

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